Human Mast Cell Sensitization with IgE Increases miRNA-210 Expression

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Keywords

miRNA-210 · Human mast cells · IgE sensitization · Allergen activation · Histamine · Prostaglandin D₂

Abstract

Background: MicroRNAs (miRNAs) represent important post-transcriptional regulators with a dynamic expression profile during health and disease. **Objectives:** We explored the miRNA profile of human mast cells (MCs) during sensitization with IgE, during activation through IgE, and related it to prostaglandin D₂ synthesis and histamine release. **Method:** We investigated the expression pattern of 762 miRNAs during the IgE-mediated sensitization and activation of MCs cultured from CD133+ stem cells that were isolated from allergic asthmatic patients and nonatopic controls. **Results:** IgE-mediated sensitization increased the expression of miRNA-210 eight-fold. This increase was sustained during IgE-mediated MC activation. Furthermore, we confirmed the increase of the miRNA-132/212 cluster after MC activation. Predicted target genes of miRNA-210/132/212 were enriched in several pathways known to be involved in MC activation. Histamine release was significantly higher in MCs from allergic patients when compared to controls, and a number of miRNAs correlated with histamine release and

prostaglandin D₂ synthesis during MC activation. **Conclusion:** The miRNAs and analysis presented here can help to elucidate the role of miRNAs in mediator release during MC activation. We speculate that miRNA-210 could be important in MC sensitization that leads to allergic symptoms.

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Introduction

Sensitization with and cross-linking of IgE activates mast cells (MCs) to release the mediators, prostaglandin D₂ (PGD₂), cysteinyl leukotrienes, and histamine, that cause asthma and other allergic diseases. MicroRNAs (miRNAs) represent a hitherto little-explored layer of control of MCs but have been found in the MC line HMC-1 and in exosomes released from these cells [1]. The increase in the miR-132/212 cluster during MC activation has been reported [2, 3]. We cultured, sensitized, and activated MCs from blood of asthmatic patients and controls [4], and measured histamine release, PGD₂ synthesis, and miRNA profiles at each of these stages to explore the spectrum of expressed miRNAs and their relationship to the mediators relevant for distinct stages of allergic response.

Table 1. Most abundant miRNAs found in both HMCs and AMCs, listed with Δ Ct values

	Δ Ct					
	HMC			AMC		
	resting	sensitized	activated	resting	sensitized	activated
hsa-miR-223	-0.41	-0.42	-0.15	-0.70	-0.74	-0.64
hsa-miR-24	0.66	0.55	0.43	0.85	0.53	0.40
hsa-miR-628-5p	0.86	0.52	0.97	2.60	2.40	1.66
hsa-miR-222	1.08	1.20	0.73	1.20	1.15	0.95
hsa-miR-191	1.74	1.70	1.34	1.69	1.57	1.55
hsa-miR-1274B	2.20	2.17	2.07	2.50	2.36	2.34
hsa-miR-720	2.29	2.16	2.28	2.76	2.70	2.06
hsa-miR-30c	2.67	2.59	2.87	2.84	2.34	2.55
hsa-miR-21	2.87	2.85	2.96	2.99	2.28	2.69
hsa-miR-146b-5p	2.93	2.75	2.27	2.86	2.65	2.65
hsa-miR-484	3.13	3.06	2.83	3.05	2.99	2.89
hsa-miR-106a	3.25	3.47	3.25	3.35	3.19	3.23
hsa-miR-19b	3.31	3.44	3.14	3.36	3.11	3.23
hsa-miR-146a	3.53	3.35	2.73	3.49	3.21	2.96
hsa-miR-17	3.65	3.57	3.43	3.45	3.44	3.36
hsa-miR-16	3.72	3.65	3.47	4.11	3.98	3.86
hsa-miR-142-3p	3.89	3.65	3.60	3.90	3.34	3.59
hsa-miR-30b	3.95	3.80	3.88	3.89	3.55	3.73

Lower Δ Ct values equal higher expression.

Materials and Methods

MCs from allergic patients diagnosed with rhinitis and asthma (AMCs, $n = 10$) and healthy individuals (HMCs, $n = 11$) were cultured from CD133⁺ progenitor cells isolated from human peripheral blood (online suppl. Appendix 1; for all online suppl. material, see www.karger.com/doi/10.1159/000496513). The MCs were sensitized with IgE 24 h before activation with anti-IgE [4]. MC miRNA expression of 762 miRNAs was profiled in the resting, sensitized, and activated state by real-time PCR using Taqman low-density arrays (online suppl. Material and Methods).

Results and Discussion

To examine the overall miRNA expression profile of CD133-derived MCs, highly expressed miRNAs were ranked according to their expression level in resting, sensitized, and activated AMCs and HMCs (Table 1). For both groups, and in all states, an almost complete overlap was observed, and miR-223 was distinctly the most abundant miRNA. Interestingly, miR-223 is known to be involved in and specific to hematopoiesis and is important for granulocyte differentiation. Downregulation of miR-223 can promote degranulation in RBL-2H3 MCs [5]. Inhibition of MC degranulation by miR-21 was observed in

another study [6], while miR-146a was involved in MC survival [7]. Furthermore, the highly expressed miR-142-3p enhances Fc ϵ RI-mediated degranulation in LAD2 MC [8]. However, as none of these miRNAs were differentially expressed in AMCs and HMCs or in the various MC states, they may contribute to the overall MC expression profile independent of activation state.

Differential expression analysis using linear models for high-throughput data on the R *limma* framework revealed that miR-210 was upregulated 8-fold in sensitized and activated AMCs and HMCs compared to those in the resting state (Fig. 1a–c). Furthermore, the miR-132/212 cluster was upregulated 2-fold in activated AMCs and HMCs compared to in the resting state (Fig. 1a, d, e; online suppl. Appendix 2). It is noteworthy that the increase in miR-210 preceded the increase of the previously reported miR-132/212 cluster. As such, the 8-fold increase in miR-210 may have functional relevance during MC sensitization and subsequent activation, as the increase in miR-210 was sustained during activation. It is known that miR-210 is upregulated during hypoxia, stimulates angiogenesis, induces proliferation, and decreases apoptosis [9]. In an allergic context, miR-210 expression has been shown to increase in mouse lymph nodes and T regula-

Fig. 1. Differentially expressed miRNAs in resting, IgE-sensitized and anti-IgE-activated mast cells ($n = 10$) and HMCs ($n = 11$). **a** IgE sensitization and anti-IgE activation of cultured mast cells and differentially expressed miRNAs. **b, c** miR-210 expression increased after sensitization. This increase was sustained after activation. **b** miR-210 ΔC_t expression values in resting AMC and HMCs. **c** Fold changes of miR-210 in sensitized and activated AMC and HMCs compared to in resting AMC and HMCs. Fold changes were calculated using the $\Delta\Delta C_t$ method where the final values were determined using $2^{-\Delta\Delta C_t}$. Fold changes of miR-212 (**d**) and miR-132 (**e**) in sensitized and activated AMC and HMCs compared to in resting AMC and HMCs. Error bars reflect SEM. * $p < 0.05$, adjusted for multiple comparisons (Benjamini-Hochberg adjusted), as implemented in the *limma* Bioconductor package for R. **f** Experimentally validated targets of miR-210, miR-212 and miR-132, retrieved from miRTarBase. **g** miRNA gene targets were used as input for functional pathway enrichment analysis. Significantly enriched REACTOME pathways with an adjusted p value < 0.05 were visualized according to p value.

Fig. 2. Histamine release (HR) and prostaglandin D₂ (PGD₂) synthesis in AMCs ($n = 10$) and HMCs ($n = 11$) after anti IgE-activation. **a** HR was significantly increased in AMCs compared to in HMCs. HR represents a percentage of the total histamine content of the sample. The spontaneous HR, without anti-IgE activation, amounted to 1–2% in all experiments. **b** No difference in PGD₂ synthesis was observed between AMCs and HMCs. For AMCs (**c**), a significant negative correlation between PGD₂ synthesis and HR was observed, while there was no significant correlation found for HMCs (**d**). **e–i** The expression of 5 miRNAs was significantly correlated to HR when including data from both AMCs and HMCs.

Higher expression levels of miR-650, miR-15a, and miR-186 correlated with lower HR, while lower expression of miR-106b and miR-18a correlated with higher HR. **j–l** Three miRNAs were significantly correlated with PGD₂ synthesis. Higher expression level of miR-103 correlated with lower PGD₂ levels, while increased expression of miR-338-5p and miR-629 correlated with higher levels of PGD₂. **a, b** Error bars reflect SD. * $p < 0.05$ from a two-tailed Student t test. **c–l** The Pearson and Spearman correlations were calculated along with the corresponding two-tailed p values. A correlation line was fitted using linear regression and includes 95% confidence bands.

tory cells following dermal chemical sensitization [10], and miR-210 is upregulated in exosomes released by the HMC-1 cell line [11].

To pinpoint the functional impact of the miR-210/132/212 upregulation during MC activation, human gene targets with strong experimental evidence were retrieved from miRTarBase and used as input for REACTOME pathway enrichment analysis (Fig. 1f, g). Several pathways important for MC activation were among the top enriched pathways, including interleu-

kin and cytokine signaling. More specifically, these miRNAs may be involved in high-affinity IgE receptor (FcεRI)-mediated MC activation by modulating non-receptor tyrosine kinases (NRTKs) involved in MC activation, e.g., Bruton's tyrosine kinase (Btk) and the tyrosine protein-kinases Lyn and Fyn, to control receptor signaling events. Downstream mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase-protein kinase B (PI3K/AKT) signaling are also enriched. Thus, it is plausible that these miRNAs could be

involved in FcεRI-mediated MC activation affecting NRTK signaling transduction through PI3K-AKT and MAPK signaling before MC degranulation and interleukin/cytokine generation. Furthermore, the BTK gene is a direct target of miR-210 [12]. In line with this, miR-210 has previously been shown to increase expression of interleukin (IL)-1α and tumor necrosis factor (TNF)-α in HL1 cardiomyocytes [13]. In MCs, IL-1α leads to the upregulation of IL-3, IL-5, IL-6, IL-9, and TNF-α [14]. While this was not linked to an increase in miR-210 in this study, this could point to a possible pro-inflammatory response induced by miR-210 during sensitization. Thus, miR-210 could be functionally important to prime MCs for activation following sensitization. Antagonizing miR-210 during sensitization would be interesting, to observe possible changes in subsequent MC activation.

MC degranulation was evaluated by histamine release (HR) and PGD₂ synthesis. HR was significantly elevated in AMCs compared to in HMCs, while no difference was observed for PGD₂ synthesis (Fig. 2a, b). Thus, the AMC degranulation response to anti-IgE activation was more pronounced than in HMCs, indicating a difference in the MCs isolated from allergic asthmatics and controls. Furthermore, a significant inverse relationship between HR and PGD₂ synthesis was detected in AMCs (Fig. 2c, d), suggesting that a high concentration of PGD₂ may inhibit MC degranulation. In a global correlation analysis, the expression of several MC miRNAs correlated significantly with PGD₂ and HR. However, many of these miRNAs had a very low expression level (>30 Ct; online suppl. Appendix 3, 5), and may thus have limited functional impact. When looking at correlations where miRNA expression was <30 Ct, 5 significant miRNA-HR correlations (miR-650, miR-15a, miR-106b, miR-186, and miR-18a) and 3 significant miRNA-PGD₂ correlations were found (miR-338-5p, miR-103, and miR-629) (Fig. 2e–l). By target prediction and functional enrichment analysis, IL-4 and SMAD2/3/4 were among the pathways enriched in HR-miRNA correlations, while phosphatidylinositol-3,4,5-triphosphate/AKT (PIP3/AKT), tumor protein p53 (TP53), and RTK signaling were among the pathways enriched in the PGD₂-miRNA correlations (online suppl. Appendix 4, 6). Accordingly, these miRNAs and affected pathways may be important for distinguishing the pathways involved in determining the relative levels of HR degranulation and PGD₂ synthesis. Modulation of these miRNAs could provide targets to therapeutically target and modulate HR and PGD₂ synthesis.

Conclusion

In conclusion, we show for the first time that the expression of the biologically important miR-210 was increased by MC sensitization and activation, while we also observed the previously reported increase in miR-132/212 expression during activation. Target genes of miR-210/132/212 were enriched in several pathways known to be involved in MC activation, indicating that these miRNAs have the potential to modulate MC activation. HR was increased in activated AMCs compared to in HMCs, and an inverse correlation was found between HR and PGD₂ release in AMCs. Lastly, several miRNAs showed a significant correlation with HR and PGD release. The miRNAs and subsequent analysis presented here may help to elucidate the role of miRNAs in MC activation and mediator release. We speculate that miR-210, in particular, could be important for MC sensitization and, as such, prime MCs for subsequent activation.

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Statement of Ethics

The study was approved by the regional ethics committee, and patients gave informed consent before donating blood.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

OS, PF and HJH conceived the study. HJH and TS recruited patients. PF and MK performed experiments. JJ and SL performed data analysis. JJ and HJH interpreted data and wrote the manuscript.

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